REMARKS

Applicant has made a significant contribution to the field of cancer therapy by identifying an anti-tumor portion of the ANUP protein. The N-terminal fragment was found to possess significant anti-tumor activity.

Applicant thanks the Examiner for his helpful comments. The claims have been amended in accordance with the Examiner's suggestions and to be commensurate with the scope of the originally-filed disclosure and with Applicant's fundamental contribution the art.

Upon entry of this amendment, claims 4-9 and 15-18 are pending in the instant application. Claims 4-8 and 15 have been amended, claims 15-18 have been added and claims 10-14 have been cancelled herein without prejudice or disclaimer. Applicant reserves the right to prosecute that subject matter, as well as the originally presented claims, in continuing applications. Support for the new claims and claim amendments presented herein is found throughout the specification and in the claims as originally filed. For example, support for the polypeptides consisting of the amino acid sequence of SEQ ID NO: 1, as recited by amended claims 4-7, is found at least at page 2, lines 2-4; at page 2, lines 14-32; and at page 3, lines 20-29 of the as-filed specification. Support for the methods of killing a tumor cell, as recited by amended claims 8-9, is found at least at page 2, lines 4-7; at page 2, line 37 through page 3, line 5; at page 3, lines 14-17 and at page 4, line 1 through page 5, line 12 of the as-filed specification. Support for the methods of activating an anti-tumor polypeptide, as recited by amended claim 15, is found in the specification at least at page 2, lines 11-14 and at page 5, lines 1-5 of the as-filed specification.

Accordingly, no new matter has been added by this amendment.

1. Objections to the Specification

The Examiner has objected to the as-filed specification and has reminded Applicant of the preferred arrangement for the specification.

Applicant submits herewith a substitute specification under the provisions of 37 CFR 1.125(b) and (c). In the substitute specification submitted herewith, Applicant has amended the specification in accordance with the preferred arrangement of the specification. In particular,

Applicant has re-arranged certain sections of the as-filed specification, but Applicant has not added any additional information. Accordingly, Applicant requests that the Examiner replace the as-filed specification with the substitute specification submitted herewith.

The Examiner has also objected to the Abstract of the Disclosure. In particular, the Examiner has asserted that the Abstract should be presented on a separate sheet of paper, and that any references cited in the Abstract should contain matched parentheses.

Applicant notes that in the substitute specification submitted herewith, the Abstract has been presented on a sheet of paper that is separate from any other part of the specification. In addition, Applicant has removed all references from the Abstract. Accordingly, this objection should be withdrawn.

The Examiner has also indicated that page 1 of the as-filed specification was not numbered, and moreover, it provides information that is neither "necessary nor desirable".

Applicant notes that all pages of the substitute specification submitted herewith have been numbered. In addition, all information provided on page 1 of the as-filed specification, excluding the Title of the Invention, has been deleted in the substitute specification submitted herewith. Accordingly, the Examiner should withdraw these objections.

2. Claim Rejections Under 35 U.S.C. § 112, First Paragraph (Written Description)

Claims 4-11 and 15:

Claims 4-11 and 15 have been rejected under 35 U.S.C. §112, first paragraph for lack of written description. According to the Examiner, "it does not appear that support exists for a peptide that comprises SEQ ID NO: 1". (Office Action, p. 3).

The specification discloses a purified ANUP protein and an N-terminal peptide of the protein, the peptide containing the amino acid sequence of SEQ ID NO: 1, as well as methods of using the peptides to promote apoptosis and methods of activating these peptides. Support for these peptides and methods recited by the pending claims, as amended herein, is found throughout the originally filed specification. For example, support for a polypeptide containing the amino acid sequence of SEQ ID NO: 1, as recited by amended claims 4-7, is found at least at page 2, lines 2-4; at page 2, lines 14-32; and at page 3, lines 20-29 of the as-filed specification.

Support for the methods of killing a tumor cell, as recited by amended claims 8-9, is found at least at page 2, lines 4-7; at page 2, line 37 through page 3, line 5; at page 3, lines 14-17 and at page 4, line 1 through page 5, line 12 of the as-filed specification. Support for the methods of activating an anti-tumor polypeptide, as recited by amended claim 15, is found in the specification at least at page 2, lines 11-14 and at page 5, lines 1-5 of the as-filed specification. At least two examples (N-terminal fragment of ANUP and ANUP protein) of compositions comprising the amino acid sequence of SEQ ID NO:1 are disclosed in the specification. Accordingly, Applicant believes that the pending claims are supported by the as-filed specification, and this rejection should be withdrawn.

Claims 10-11:

Claims 10-11 have been further rejected under 35 U.S.C. §112, first paragraph for lack of written description. Applicant notes that claims 10 and 11 have been cancelled herein. Thus, any rejection of these claims has been rendered moot and should be withdrawn.

Claim 15:

Claim 15 has also been further rejected under 35 U.S.C. §112, first paragraph for lack of written description. According to the Examiner, "it does not appear that there is descriptive support any other detergent or for detergents in general". (Office Action, p. 4).

Applicant notes that claim 15 has been amended to recite methods of activating an anti-tumor polypeptide by contacting the polypeptide with sodium dodecyl sulfate, wherein the anti-tumor activity of the polypeptide is activated after the polypeptide has been contacted with sodium dodecyl sulfate, wherein the polypeptide contains of the amino acid sequence of SEQ ID NO:1, and wherein the activated anti-tumor polypeptide promotes apoptosis in a tumor cell.

Thus, the methods of claim 15, as amended herein, do not use *any unspecified* detergent. Rather, polypeptides activated according to these methods are contacted with sodium dodecyl sulfate, as described throughout the specification as originally filed. (*See e.g.*, as-filed specification at page 4, lines 12-13 and at page 5, lines 9-12). Thus, the methods recited by claim are supported by the as-filed specification, and the Examiner should withdraw this rejection.

3. Claim Rejections Under 35 U.S.C. § 112, First Paragraph (Enablement)

Claims 8-11 have been rejected under 35 U.S.C. §112, first paragraph for lack of enablement. According to the Examiner, these claims encompass both *in vitro* and *in vivo* methods of killing a tumor cell, and "enablement is lacking, at least for the case of achieving the 'contacting' by administering the peptide to a tumor-bearing mammal." (Office Action, p. 4).

Claims 8-9 have been amended herein in accordance with the claim amendments suggested by the Examiner at page 5 of the Office Action. In particular, claims 8-9 have been amended herein to recite methods of killing a tumor cell by contacting the tumor cell with a polypeptide containing of the amino acid of SEQ ID NO:1 for a time and under conditions effective to kill the tumor cell by apoptosis. Such methods are described at least at page 4, line 1 through page 5, line 12 of the as-filed specification in such a way as to allow one of ordinary skill in the art to make and/or use these claimed methods. The data provided in the as-filed specification are representative of standard *in vitro* cell-based assays, which are used by those skilled in the art to evaluate performance of a composition *in vivo*. Results of such *in vitro* assays are generally regarded as predictive of performance *in vivo*.

The compositions have now also been tested *in vivo* using a well-known animal model for clinical cancer. Both an N-terminal polypeptide of ANUP protein and the ANUP protein (purified from plasma) were tested to evaluate tumor killing in nude mice. Applicant submits herewith a Declaration Under 37 C.F.R. §1.132 of Paul DiTullio, a researcher in the field cell biology. This Declaration presents data demonstrating that ANUP peptide fragments kill tumor cells (thereby reducing tumor load) in an art-recognized tumor model. Human cervical cancer cells were transplanted into nude mice, and peptide was administered to the tumor-bearing mammal. (See DiTullio Declaration). The data obtained using the animal model confirms that the *in vitro* data disclosed in the as-filed specification is predictive of anti-tumor activity of the peptides in a tumor-bearing animal. As evidenced by the data presented in the Declaration, the claimed compositions and methods predictably lead to tumor cell killing.

Accordingly, the as-filed specification is enabling for the scope of the amended claims, and this rejection should be withdrawn.

4. Claim Rejections Under 35 U.S.C. § 112, second paragraph

Claims 5-11 and 15 have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. Claims 10 and 11 have been cancelled. The remaining claims are addressed below.

Claim 5:

The Examiner has asserted that claim 5 is ambiguous, as the term "'agent' could refer to a single compound, or to a mixture of compounds, or a composition." (Office Action, p. 6).

Applicant notes that claim 5 and its dependent claims, claims 6-7, have been amended herein to recite "anti-tumor polypeptides" comprising an apoptosis-inducing concentration of a polypeptide comprising the amino acid sequence of SEQ ID NO:1. Thus, these claims are directed to a single compound (*i.e.*, an "anti-tumor polypeptide"). Accordingly, claims 5-7 are not ambiguous, and this rejection should be withdrawn.

Claim 6:

The Examiner has indicated a minor typographical error, the term "polyeptide," in claim 6. Applicant has amended claim 6 to recite the term "polypeptide". Accordingly, Applicant requests that the Examiner withdraw this rejection.

Claim 8:

The Examiner has objected to claim 8 as being indefinite "as to the process steps and endpoint" of the recited methods. The Examiner has suggested amending claim 8 to include the limitation "for a time and under conditions effective to promote apoptosis in a tumor cell." (Office Action, p. 7).

Applicant notes that claims 8-9 have been amended in accordance with the Examiner's suggested claim amendments at pages 5 and 7 of the Office Action. In particular, claims 8-9 have been amended herein to recite methods of killing a tumor cell by contacting the tumor cell with a polypeptide comprising the amino acid of SEQ ID NO:1 for a time and under conditions effective to promote killing by apoptosis in said tumor cell. Accordingly, amended claims 8 and 9 recite the process steps and endpoint of the claimed methods of killing a tumor cell, and this rejection should be withdrawn.

Claim 15:

With regard to claim 15, the Examiner has asserted that this claim is dependent on a non-elected claim. Applicant notes that claim 15 has been re-written as an independent claim, in accordance with the Examiner's suggestion on page 7 of the Office Action. Accordingly, Applicant requests that the Examiner withdraw this rejection.

The Examiner has also asserted that claim 15 is indefinite "as to the manifestations of the activation" of the recited methods of activating an anti-tumor polypeptide. (Office Action, page 7).

Applicant notes that claim 15 has been amended to recite methods of activating an anti-tumor polypeptide by contacting the polypeptide with sodium dodecyl sulfate, wherein the anti-tumor activity of the polypeptide is activated after the polypeptide has been contacted with sodium dodecyl sulfate, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:1, and wherein the activated anti-tumor polypeptide promotes apoptosis in a tumor cell.

Thus, the activated anti-tumor polypeptides produced by the methods of claim 15 promote apoptosis in a tumor cell, wherein the promotion of apoptosis in a tumor cell is the "manifestation of the activation" of a polypeptide comprising the amino acid sequence of SEQ ID NO: 1. Accordingly, the methods of activating a peptide recited by amended claim 15 are not indefinite as to the manifestations of activation, and Applicant requests that the Examiner withdraw this rejection.

CONCLUSION

On the basis of the foregoing amendments, Applicant respectfully submits that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

for Jennifer Aiken Reg No. 53,097

Ingrid A. Beattie, Reg. No. 42,306
Attorney for Applicant
c/o MINTZ, LEVIN
One Financial Center
Boston, MA 02111
Telephone (617) 542 6000
Fax (617) 542 2241

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THE USE OF THE ACTIVATED N-TERMINAL SIXTEEN AMINO ACID PEPTIDE OF THE ANTINEOPLASTIC PROTEIN (ANUP) AS A PHARMACOLOGICALLY ACTIVE ANTI-TUMOR AGENT

~~Inventor:~~ ~~Nathan H. Sloane~~
~~1842 Brookside Drive~~
~~Germantown, TN 38138~~

~~Assignee:~~ ~~Antitumor Research Products, Inc.~~
~~1842 Brookside Drive~~
~~Germantown, TN 38138~~

~~References Cited~~

~~U.S. Patent Documents~~

~~4,359,415 11/1982 Sloane~~
~~4,559,325 12/1985 Burzynski~~
~~5,008,372 4/1991 Wellner~~
~~5,928604 3/1994 Sloane~~

~~U.S. Application Number 08/641,905 05/02/96 Sloane~~

~~OTHER PUBLICATIONS~~

~~Sloane et al. Biochemical Journal (1986), 234, pp. 355-362.~~
~~Pottathil et al., Cancer Res. Therapy and Control (1990), 1, pp. 193-198.~~
~~Struve et al. Cancer Res. Therapy and Control (1990) 1: pp. 225-230.~~
~~Ridge and Sloane, Cytokine (1996) 8 pp. 1-5~~
~~Sloane and Davis, Tumor Targeting (1996) 2 pp 322-326.~~

FIELD OF THE INVENTION

The present invention relates to the use of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) as a pharmacologically active antitumor agent. ~~The peptide is about 50% as active as the protein per se but only about one-tenth of the weight of the peptide is equivalent in activity of the protein (ANUP) on a molar basis (ca 10^{-9} M).~~

BACKGROUND OF THE INVENTION

The Antineoplastic Protein (ANUP) kills tumor cells. The protein (ANUP) in the purified state has been implicated in regression of both HeLa (human cervical tumor all line) and KB (human laryngeal cell line) implanted in nude mice.

SUMMARY OF THE INVENTION

The present invention describes the pharmacologically active anti-tumor activity of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP).

The 16 amino acid peptide is approximately one-half as active as the protein on a molar basis utilizing the human breast tumor cell line (MDA 231). However, only about one-tenth of the weight of the peptide is required when compared to the amount of protein for equivalent activity against the human breast tumor cell line. Both the protein and the peptide exert their action by killing tumor cells (apoptosis) since electron microscopy studies showed complete degradation of the cells (Struve et al. Cancer Res. Therapy and Control (1990) 1: pp 225-230).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) as a pharmacologically active antitumor agent. The peptide is about 50% as active as the protein per se but only about one-tenth of the weight of the peptide is equivalent in activity of the protein (ANUP) on a molar basis (ca 10^{-9} M).

DESCRIPTION OF THE PREFERRED EMBODIMENT

The 16 Amino Acid Peptide

The synthetic hexadeca peptide (16 L-amino acids) has the following sequence:

1.	~~Pyroglu~~ Glu	E	9.	Glu	E
2.	Leu	L	10.	Pro	P
3.	Lys	K	11.	Met	M
4.	Cys	C	12.	Thr	T
5.	Tyr	Y	13.	Ser	S
6.	Thr	T	14.	Ala	A
7.	Cys	C	15.	Ala	A
8.	Lys	K	16.	Cys	C (SEQ ID NO: 1)

The peptide was synthesized by Research Genetics Inc., in Huntsville, AL 35801; the peptide was pure as shown by HPLC (high performance liquid chromatography) and the molecular weight was check by mass spectrometry (MS).

The 16 amino acid peptide representing the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) is a highly active pharmacologically antitumor agent. The 16 amino acid peptide is about 50% as active as antitumor agent compared to the antitumor active as the protein (ANUP) per se when tested as a tumor killer agent (in vitro) utilizing human breast tumor cell line (MDA 231). The protein (ANUP) in the purified state also shows regression of both HeLa (human cervical tumor all line) and KB (human laryngeal cell line) implanted in nude mice (Sloane, Davis Tumor Targeting (1996) 2, pp 322-326). The nonapeptide is about 10% as active compared to the antineoplastic protein (ANUP) in the human breast tumor cell line in vitro assay system. Both peptides, the 9 amino acid peptide and the 16 amino acid peptide require presence of the detergent sodium dodecyl sulfate to activate the peptides for full pharmacological antitumor activity.

EXAMPLES

Example 1: The pharmacological anti-tumor activity of the 16 amino acid peptide (P_{16})

The antitumor activity of the peptide (P_{16}) was assayed against the human breast tumor cell line (MDA 231) and its activity was compare to the in vitro antitumor effect of the "pure" protein (ANUP).

The assay for the pharmacological antitumor activities were performed as follows utilizing 96 well plates --

20,300 - 30,000 human breast tumor cells in L-15 medium (200 ~~ul~~ µl) containing 2.5 % fetal calf serum and 100 ~~ug~~ µg ~~gestamycin~~ gentamycin per ml (complete medium) were incubated at 37° in air for 120 hours; after this incubation period 50 ~~ul~~ µl of serially diluted P_{16} and ANUP were added to each well. The serial dilutions were prepared as follows: 2 mg each (the P_{16} and ANUP) were dissolved in 2 ml of complete medium containing 0.5% sodium dodecyl sulfate (SDS). The solutions were diluted in complete medium containing 0.05% SDS to a concentration of 350 ~~ug~~ µg per ml.

Dilution plates were prepared as follows:

100 ~~ul~~ µl of complete medium were added to each well and 50 ~~ul~~ µl of diluted P_{16} and ANUP were added to each well in row A thus 1.3 dilution was accomplished; 50 ~~ul~~ µl were serially diluted in the 100 ~~ul~~ µl of medium in rows B through H. Thus the range of concentrations were from 6 ~~ug~~ µg to 2 mg when 50 ~~ul~~ µl each dilution series were added to the 200 ~~ul~~ µl of the complete medium containing the MDA cells. The plates were incubated for an additional 96-120 hours. The medium was poured off and after a 90-minute incubation with 50 ~~ul~~ µl neutral red dye (0.5 ml neutral red (0.25% ethanol (0.6 ml) diluted 5.5 saline - 0.16 mm HCl) the cells were washed twice with PBS (phosphate buffer saline) at room temperature. The concentration of living cells (since only living cells absorb the dye) was determined after adding 100 ~~ul~~ µl lysing buffer (50% ethanol in 0.05 m NaH_2PO_4) the concentration of neutral red released in each well was determined using a Dynetech plate reader set at 550 mm. A unit of activity was defined as the concentration of ANUP and P_{16} for 50% killing.

Under these assay conditions the 50% end points were as follows:

ANUP 0.1 ~~ug~~ μg /well = 1.25×10^{-8} M

P_{16} 0.0 ~~ug~~ μg /well = 2.2×10^{-8} M

Thus, P_{16} is about 50% as active as ANUP on a molar basis; whereas on a weight basis only one tenth of the peptide weight is equal in activity 10 times the weight of the protein (ANUP).

In the absence of SDS neither the peptide nor the protein showed any antitumor activity. Thus the detergent is probably necessary to form the correct geometrical shape for activity as described by Sloane and Davis Tumor Targeting (1996) 2, 322-326. The data utilizing P_{16} as an antitumor agent against the human breast tumor cell line (MDA 231) are as follows:

	Fraction of the Activity relative to ANUP
P_{16} no SDS	± no Activity
P_{16} + 0.005% SDS	0.04
P_{16} + 0.02% SDS	0.50
P_{16} + 0.05% SDS	0.50

~~ABSTRACT~~

~~The 16 amino acid peptide representing the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) is a highly active pharmacologically antitumor agent. The 16 amino acid peptide is about 50% as active as antitumor agent compared to the antitumor active as the protein (ANUP) per se when tested as a tumor killer agent (in vitro) utilizing human breast tumor cell line (MDA 231). The protein (ANUP) in the purified state also shows regression of both HeLa (human cervical tumor all line) and KB (human laryngeal cell line) implanted in nude mice (Sloane, Davis Tumor Targeting (1996) 2, pp 322-326. The nonapeptide is about 10% as active compared to the antineoplastic protein (ANUP) in the human breast tumor cell line in vitro assay system. Both peptides, the 9 amino acid peptide and the 16 amino acid peptide require presence of the detergent sodium dodecyl sulfate to activate the peptides for full pharmacological antitumor activity.~~

~~The ANUP N-terminal 16 amino acid peptide contains the following sequence (as L-Amino Acids):~~

~~1. Pyroglu~~
~~2. Leu~~
~~3. Lys~~
~~4. Cys~~
~~5. Tyr~~
~~6. Thr~~
~~7. Cys~~
~~8. Lys~~
~~9. Glu~~
~~10. Pro~~
~~11. Me~~
~~12. Thr~~
~~13. Ser~~
~~14. Ala~~
~~15. Ala~~
~~16. Cys (SEQ ID NO: 1)~~

~~The use of the N-terminal Sixteen Amino Acid Peptide as a Pharmacologically Active Anti-tumor Agent.~~

~~BACKGROUND OF THE INVENTION~~

THE USE OF THE ACTIVATED N-TERMINAL SIXTEEN AMINO ACID PEPTIDE OF THE ANTINEOPLASTIC PROTEIN (ANUP) AS A PHARMACOLOGICALLY ACTIVE ANTI-TUMOR AGENT

ABSTRACT

The invention provides a 16 amino acid peptide representing the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP). The 16 amino acid peptide provided is a highly active pharmacologically antitumor agent.